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# T cell vaccination in multiple sclerosis: results of a preliminary study



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**Abstract** Myelin basic protein (MBP)-reactive T cells are potentially involved in the pathogenesis of multiple sclerosis (MS), and can be depleted by subcutaneous inoculations with irradiated autologous MBP-reactive T cells (T cell vaccination). This preliminary open label study was undertaken to evaluate whether depletion of MBP-reactive T cells would be clinically beneficial to patients with MS. Fifty-four patients with relapsing-remitting (RR) MS (n=28) or secondary progressive (SP) MS (n=26) were immunized with irradiated autologous MBP-reactive T cells and monitored for changes in rate of relapse, expanded disability scale score (EDSS) and MRI lesion activity over a period of 24 months. Depletion of MBP-reactive T cells correlated with a reduction (40%) in rate of relapse in RR-MS patients as compared with the pre-treatment rate in the same cohort. However, the reduction in EDSS was minimal in RR-MS patients while the EDSS was slightly increased in SP-MS patients over a period of 24 months. Serial semi-quantitative MRI examinations suggest stabilization in lesion activity as compared with baseline MRI. The findings suggest some potential clinical benefit of T cell vaccination in MS and encourage further investigations to evaluate the treatment efficacy of T cell vaccination in controlled trials.

**Key words** multiple sclerosis · T cell vaccination

## Abbreviations

EAE – experimental autoimmune encephalomyelitis
PHA – phytohemagglutinin
PBMC – peripheral blood mononuclear cells
MBP – myelin basic protein
MRI – magnetic resonance imaging
MS – multiple sclerosis
RR-MS – relapsing-remitting MS
SP-MS – secondary progressive MS

## Introduction

There is growing evidence that autoimmune T cell responses to myelin antigens, including myelin basic protein (MBP), may be engaged in the pathogenesis of multiple sclerosis (MS) [17]. MBP-reactive T cells are found to undergo *in vivo* activation and occur at high precursor frequency in the blood and cerebrospinal fluid of patients with MS [1, 4, 26]. These MBP-reactive T cells produce pro-inflammatory Th1 cytokines (IL-2, TNF-α and γ-interferon) and are thought to facilitate myelin-destructive inflammation in the central nervous system [15, 16]. It has been shown that MBP-reactive T cells can induce experimental autoimmune encephalomyelitis

(EAE), an animal model for MS [2]. EAE can also be prevented or cured by repeated inoculations with MBP-reactive T cells that have been inactivated by chemical treatment or irradiation, a treatment procedure termed T cell vaccination [3]. It has been demonstrated that T cell vaccination induces regulatory immune responses comprised of anti-idiotypic T cells and anti-ergotypic T cells, which contribute to the treatment effects on EAE and other experimental autoimmune disease models [9, 10].

T cell vaccination has advanced recently to clinical trials in patients with MS based on the hypothesis that depletion of MBP-reactive T cells may improve the clinical course of the disease. In a pilot clinical trial, we demonstrated that vaccination with irradiated autologous MBP-reactive T cell clones elicited CD8+ cytolytic T cell responses that specifically recognized and lyzed MBP-reactive T cells used for vaccination [11, 25]. Three subcutaneous inoculations with irradiated MBP-reactive T cell clones resulted in depletion of circulating MBP-reactive T cells in patients with MS. Depletion of MBP-reactive T cells by T cell vaccination appeared to correlate with clinical improvement, as evidenced by a reduction in rate of relapse, expanded disability scale score (EDSS) and MRI lesion activities in relapsing-remitting patients [11]. Although no conclusion could be made from the pilot trial owing to the limited number of patients studied, the excellent safety profile and the potential clinical benefit encouraged further clinical investigations. This preliminary study was undertaken to investigate whether depletion of circulating MBP-reactive T cells would be clinically beneficial to patients with MS. Twenty-eight patients with relapsing-remitting MS (RR-MS) and 26 patients with secondary progressive MS (SP-MS) were included in this open-label study. Patients received three subcutaneous injections of irradiated autologous MBP-reactive T cells and were monitored for changes in rate of relapse, EDSS and MRI lesion activities over a period of 24 months. The results were compared with pre-study values in a self-paired fashion.

## Materials and methods

### Patients and the study design

Fifty-four patients with MS were enrolled in this study. The inclusion criteria were clinically definite MS for at least two years, baseline EDSS of 1.5 to 6.5 for RR-MS and 4.0 to 8.0 for patients with SP-MS, and at least one exacerbation in the past two years prior to study entry for the RR-MS cohort. Approximately 25% of the patients failed previously to respond to or tolerate treatment with beta-interferon or Glatiramer, and the remaining patients had not been treated with these agents at least three month prior to entry and throughout the study. The patients had not taken any immunosuppressive drugs, including steroids, at least three months prior to enrolling in the study. Steriods were permitted during the study if an exacerbation occurred. Symptomatic treatments for fatigue, spasticity and bladder complaints were not prohibited. Informed consent was obtained from the patients after explaining the experimental procedures. The protocol was approved by the Institutional Human Subject Committee at Baylor College of Medicine.

All patients were given three subcutaneous injections with irradiated autologous MBP-reactive T cells, and observed for time to onset of confirmed progression of disability, EDSS, rate of relapse and MRI lesion activities. The results were compared with the patient's own pre-treatment course. Time to progression was determined by an increase of at least 1.0 on the EDSS [13] persisting for at least 3 months. On-study exacerbations were defined by the appearance of new neurological symptoms or worsening of pre-existing neurological symptoms lasting for at least 48 hours, accompanied by objective change on neurological examination (worsening of at least 0.5 point on EDSS). Patients were instructed to report events between the scheduled regular visits, and were examined by a neurologist if symptoms suggested an exacerbation. Safety assessments included adverse events, vital signs and physical examinations at regular visits.

### Estimation of the frequency of MBP-reactive T cells in the blood

The method was described previously [11, 25, 26]. In each case, the material used for cell processing and cell culture was strictly autologous. Peripheral blood mononuclear cells (PBMC) were prepared from heparinized venous blood by Ficoll gradient separation. PBMC were plated out at 200,000 cells/well in the presence of MBP (40 μg/ml) and two synthetic MBP peptides corresponding to the immunodominant regions (residues 83–99 and residues 151–170), respectively, at a concentration of 20 μg/ml. The cell number per well had been predetermined as an optimal cell density to detect MBP-reactive T cells. Seven days later, all cultures were restimulated with autologous PBMC pulsed with MBP and the MBP peptides, respectively. Pulsing of PBMC was carried out by incubating PBMC with the peptides or MBP (100 μg /ml) at 37°C for three hours. Pulsed PBMC were then irradiated at 4,000 rads before use. After another week, each culture was examined for specific proliferation to MBP and the MBP peptides in proliferation assays. Briefly, each well was split into four aliquots (approximately $10^4$ cells per aliquot) and cultured in duplicate with $10^5$ autologous PBMC in the presence and the absence of MBP and the MBP peptides. Cultures were kept for three days and pulsed with [$^3$H]-thymidine (Amersham, Arlington Hights, IL) at 1 μCi per well during the last 16 hours of culture. Cells were then harvested using an automated cell harvestor and [$^3$H]-thymidine incorporation was measured in a betaplate counter. A well/culture was defined as specific for the peptides when the CPM were greater than 1,500 and exceeded the reference CPM (in the absence of the antigens) by at least three times. The frequency of MBP-reactive T cells was then estimated by dividing the number of specific wells by the total number of PBMC seeded in the initial culture for each antigen [11, 25, 26]. The second precursor frequency analysis of MBP-reactive T cells was performed using the whole MBP as the antigen 2–3 months after the completion of T cell vaccination. The same method of calculation was used consistently to compare the changes of the T cell frequency throughout the study.

### Generation of MBP-reactive T cell clones

The resulting T cell lines either specific for the MBP peptides or MBP were selected for cloning. These T cell lines were CD4+/CD8 – and exhibited Th1-like phenotype producing TNF-α and γ-interferon but little or no IL-4 and IL-10. They were cloned subsequently by the limiting dilution assay [11, 25, 26]. Briefly, cells of each T cell line were plated out under limiting dilution conditions at 0.3 cell per well and cultured with $10^5$ irradiated autologous PBMC and 2 μg/ml of PHA (Sigma, St. Louis, MO). Cultures were fed every three to four days with fresh medium containing 50 IU /ml of recombinant IL-2 (Chiron, San Diego, CA). After approximately 10–12 days, growth-positive wells became visible and were tested in proliferation assays to select T cell

clones specific for the peptides of MBP. The medium used for T cell culture and cell processing was RPMI 1640 (Hyclone, Logan, UT) supplemented with 10% heat-inactivated autologous serum and 50 IU/ml of recombinant IL-2.

### T cell vaccination protocol

The protocol was similar to that used in previous clinical studies [11, 25]. Briefly, MBP-reactive T cell clones were pre-activated with PHA (1 μg/ml) in the presence of irradiated PBMC as a source of accessory cells. Cells were cultured for 5–6 days in RPMI1640 media supplemented with 10% heat-inactivated autologous serum and 50 units of rIL-2. Activated MBP-reactive T cells were subsequently washed three times with sterile saline to remove residual PHA and cell debris. After irradiation (8,000 rads, $^{60}$Co source), cells were resuspended in 2 ml of saline and injected subcutaneously on two arms. The number of T cells used for vaccination ranged from $30 \times 10^6$ to $60 \times 10^6$ cells per injection (2–4 T cell clones used for each injection) and was chosen by an extrapolation of T cell doses effective in experimental animals on the basis of relative skin surface areas [3]. Each patient received three subcutaneous injections at two-month intervals.

### Magnetic resonance imaging studies

Magnetic resonance imaging (MRI) was performed as gadolinium-enhanced T1 images. Areas of higher signal intensity were scored in a semiquantitative fashion [14, 19]. This scoring method produced a score related to both the size and number of foci with increased signal hyperintensity. Signal hyperintensities were scored in the following regions: (i) periventricular, in the frontal and occipital region and parallel to the lateral ventricles; (ii) lobar white matter, separately in the frontal, temporal, parietal and occipital region; (iii) the basal ganglia, caudate nucleus, putamen, globus pallidus and thalamus and (iv) the infratentorial region, cerebellum, mesencephalon, pons and medulla. The lesions were graded as follows: a lesion with a diameter less than 0.5 cm was given the score of '1', between 0.5 cm and 1.0 cm as '2', between 1.0 cm and 1.5 cm as '3', between 1.5 cm and 2.0 cm as '4' and greater than 2.0 cm as '5'. The confluent lesions were measured as follows: a score of '5' is given when less than 25% of the region of interest as defined above was considered to be of abnormal signal intensity, '10' and '15' for 25% and 50% when more than 50% of the visualized region of interest was affected. These values were then added to the 'individual' lesion scores.

### Statistical analysis

The significance in the precursor frequency of MBP-reactive T cells before and 2–3 months after vaccination was analysed by Student's *t* test. Time to onset of confirmed progression was analysed using Kaplan-Meier method. The differences in the clinical variables in study patients before and after T cell vaccination were analysed with the Wilcoxon's rank-sum test.

## Results

### The depletion of MBP-reactive T cells by T cell vaccination

Fifty-four patients with RR-MS (n=28) and SP-MS (n=26) were recruited for this open-label study. The baseline clinical characteristics of the patients are shown in Table 1. Each patient received three courses of subcutaneous injections with irradiated autologous MBP-reactive T cell clones at two-month intervals. Patients were monitored for changes in the precursor frequency of MBP-reactive T cells, rate of relapse, EDSS and MRI lesion activities over a period of 24 months. The results were compared with pre-vaccination values in a self-paired manner.

**Table 1** Pre-treatment clinical characteristics of the patients

| Patient group | No. of cases | Mean age (years) | Male/ Female | Duration (years) | EDSS at entry | Relapse rate |
|---|---|---|---|---|---|---|
| RR-MS | 28 | 45±9.7 | 13/15 | 7.4±7.3 | 3.2±2.1 | 1.25 |
| SP-MS | 26 | 49±8.1 | 10/16 | 15.5±9.3 | 6.1±0.9 | |

As is shown in Fig. 1, the precursor frequency of circulating MBP-reactive T cells detected in these MS patients at baseline ($14 \times 10^{-5}$) was highly comparable with that reported in previous studies (approximately $10 \times 10^{-5}$ in peripheral blood mononuclear cells) [11, 25]. No significant difference was found in the precursor frequency of MBP-reactive T cells between RR-MS and SP-MS cohorts. More than 90% of the patients developed significant T cell responses to the immunizing T cells after the second and the third vaccination (data not shown). The T cell frequency was undetectable in 92% of patients or declined substantially in the remaining patients 2–3 months after the completion of three courses of vaccination ($14 \times 10^{-5}$ vs. $1.9 \times 10^{-5}$, $p < 0.001$). The results confirmed depletion of MBP-reactive T cells by T cell vaccination in patients with MS.

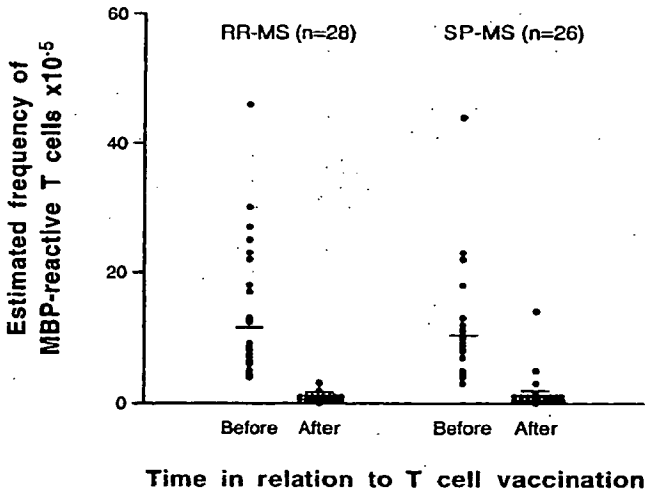


**Fig. 1** The changes in the estimated precursor frequency of circulating MBP-reactive T cells before and after vaccination.
The precursor frequency of T cells specific for MBP was estimated in all patients before and 2–3 months after the completion of T cell vaccination.

## Changes in EDSS and time to confirmed progression after depletion of MBP-reactive T cells

We attempted to address whether depletion of circulating MBP-reactive T cells by T cell vaccination would alter the clinical course of MS. Except for mild and transient erythema at the injection site seen in some patients, no adverse effects were associated with T cell vaccination, and all patients were treated in an outpatient clinic. As shown in Table 2, the mean EDSS declined slightly in patients with RR-MS (3.21 at entry vs. 3.1 at exit) over a period of 24 months after vaccination. Only one patient (3.5%) in the treated RR-MS group had progressed beyond EDSS of 2.0 within 24 months. In the SP-MS cohort, mean EDSS progressed slightly (+0.12) over a period of 24 months. Furthermore, as illustrated in Fig. 2, estimation of time to confirmed progression using the Kaplan-Meier method showed 20% progression in 18 months for both treated groups. However, progression seemed to accelerate after 18 months (12 months after the last vaccination) in both study groups.

**Table 2** Amount of sustained change in EDSS to 2 years

| Patient group | Change | EDSS | No. of cases | Percentile |
|---|---|---|---|---|
| RR-MS (n=28) | No change | 0.0 | 15 | 53.5 |
| | Better | −0.5 | 6 | 21.4 |
| | | −1.0 | 2 | 7.1 |
| | Worse | 0.5 | 4 | 14.2 |
| | | 1.0 | 0 | 0 |
| | | 1.5 | 0 | 0 |
| | | 2.0 | 1 | 3.5 |
| | Mean EDSS change | −0.11 | | |
| SP-MS (n=26) | No change | 0.0 | 12 | 46.1 |
| | Better | −0.5 | 4 | 15.3 |
| | | −1.0 | 1 | 3.8 |
| | Worse | 0.5 | 5 | 19.2 |
| | | 1.0 | 1 | 3.8 |
| | | 1.5 | 1 | 3.8 |
| | | 2.0 | 2 | 7.6 |
| | Mean EDSS change | +0.12 | | |

[a] Within-person change in EDSS from baseline to year 2.

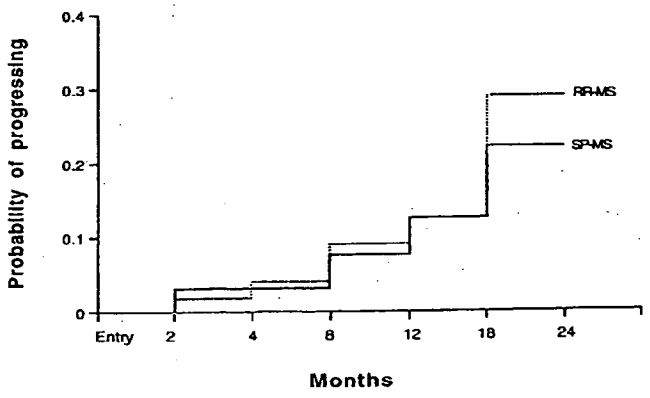


Fig. 2 Kaplan-Meier failure-time curve showing the cumulative probability of progression in relation to number of months to the onset of confirmed disability.

## Changes in rate of clinical exacerbation

As shown in Table 3, annual rate of relapse declined in patients with RR-MS after T cell vaccination, representing a 40% reduction from the baseline relapse rate. The proportion of patients exhibiting no attack was 39%. No significant difference in the rate of relapse could be found between the first year and the second year of the study. Although the rate of relapse decreased by 50% in the SP-MS cohort, it was difficult to evaluate the significance of the change as only a small number of the secondary progressive patients examined here (6/26) had a relapse during the two years prior to study entry.

## Brain lesion activities by magnetic resonance imaging examinations

Three MRI examinations (gadolinium-enhanced T1 images) were performed at entry (baseline), 12 months and at exit (24 months) to monitor changes in the brain lesion activities as an index of disease progression. Because of technical incompatibility of some scans performed at different medical centers, MRI scans from only 34 patients could be analysed. All MRI scans were evaluated by an outside neuroradiologist who was not involved in the study. A semi-quantitative scoring method used previously in our pilot study and other related studies was employed to evaluate lesion activity [11, 14, 19]. This scoring method produced a score related to both the size and number of foci with increased signal hyperintensity on T1 images. As shown in Table 4, the results revealed that in 70% of the patients examined the MRI lesion scores were either unchanged or improved as defined by a reduction of at least one point in the lesion score while the remaining 30% patients had increased lesion scores during the course of the study. As a group, the changes in the mean MRI lesion score represented a 1.2% reduction in the first year and an increase of 3.3% from the baseline MRI in the second year.

**Table 3** Frequency of clinical exacerbation

| Patient group | Annual relapse rate | No. of relapse | No. of patients | Percentile |
|---|---|---|---|---|
| RR-MS (n=28) | 1.25 (pre-study) | 0 | 11 | 39.2 |
| | 0.75 (24 months) | 1 | 4 | 14.2 |
| | | 2 | 5 | 17.8 |
| | | 3 | 5 | 17.8 |
| | | ≥4 | 3 | 10.7 |

Table 4 Mean MRI lesion score by semi-quantitative analysis and the percent change from baseline MRI.

| Patients | Baseline | 12 months (% change) | 24 months (% change) |
|---|---|---|---|
| 34 total | 14.94 | 14.76 (−1.2%) | 15.44 (+3.3%) |
| 19/34 (55%) | Unchanged | | |
| 10/34 (29%) | Increased by at least one point in MRI lesion score in 24 months | | |
| 5/34 (14%) | Decreased by at least one point in MRI lesion score in 24 months | | |

The changes, however, were not significant ($p > 0.4$). The results may suggest stabilization attributable to T cell vaccination since MRI lesions generally progress by approximately 10% on a yearly basis in non-treated RR-MS patients as documented in previous studies [8, 18]. Taken together, the findings suggest a favorable correlation between the depletion of MBP-reactive T cells by T cell vaccination and some clinical improvement in MS patients examined.

## Discussion

Although MBP-reactive T cells undergo *in vivo* activation and clonal expansion and express restricted T cell receptor V gene usage in a given individual, the T cell receptors of MBP-reactive T cells are diverse and vary between different MS patients [6, 20, 21]. Therefore, the current strategy to effectively deplete MBP-reactive T cells in MS patients requires treatment to be individualized. In agreement with the previous studies [11, 25], the present study confirms that vaccination with self MBP-reactive T cells provides a consistent and powerful means of immunizing patients in order to deplete the circulating MBP-reactive T cells. Although the mechanism underlying immune regulation induced by T cell vaccination is not completely understood, it is increasingly clear that T cell vaccination may act on multiple regulatory networks to induce CD8+ anti-idiotypic T cell responses and antibody reactions [7, 25, 27] and Th2 immune deviation [22]. In particular, these anti-idiotypic T cells induced by T cell vaccination were shown to lyse the immunizing T cells in recognition of variable regions of the T cell receptors, which represent the dominant immune regulation responsible for the depletion of MBP-reactive T cells [24]. It is conceivable that these regulatory responses induced by T cell vaccination potentially contribute to the beneficial effect of T cell vaccination in MS.

Although there is indirect evidence suggesting potential association of myelin-reactive T cells with the disease processes in MS [1, 4, 26], it has been extremely difficult, if not impossible, to establish or reject the role of myelin-reactive T cells in the pathogenesis of MS. In this regard, T cell vaccination provides a unique opportunity to assess whether depletion of myelin-reactive T cells has a beneficial impact on the clinical course of MS. The preliminary clinical trial described here suggests a favorable correlation of T cell vaccination with some improved clinical variables. First, the results indicate that depletion of MBP-reactive T cells coincided initially with slow progression in both relapsing-remitting and SP-MS cohorts. However, the disease progression seemed to accelerate 12 months after the last injection. The significance of this apparent accelerated progression is unknown, but it may be associated with a gradual decline of the immunity induced initially by T cell vaccination against MBP-reactive T cells. Indeed, in approximately 10–12% of the immunized patients, MBP-reactive T cells reappeared around that time, supporting this possibility. In some cases, the reappearing MBP-reactive T cells originated from different clonal populations that were not detected before vaccination, which was also observed in the previous studies [27]. The findings suggest that MBP-reactive T cells may undergo clonal shift or epitope spreading [20] potentially associated with the on-going disease processes. If this observation is confirmed, it may indicate the need for additional booster injections with the same or newly appearing T cell clones to maintain adequate immunity, providing important information for improving the current protocol of T cell vaccination. This possibility was explored in a recent study [5].

Annual MRI examinations revealed a slight reduction in MRI lesion activities in the first year and only a 3.3% increase in the second year. The MRI findings may represent stabilization in lesion activity in patients treated with T cell vaccination. There were favorable changes in other clinical variables, including annual rate of relapse and EDSS in vaccinated patients, suggesting a potential beneficial effect of T cell vaccination on the clinical course of MS. The results of the study are largely consistent with the findings reported in the pilot study [11]. However, in contrast to other clinical variables, the impact of T cell vaccination on clinical disability as measured by EDSS was minimal in both study groups. It may reflect the lack of sensitivity of the EDSS to measure changes over a relatively short period of time (24 months). The possibility also exists that even after the autoimmune component is removed or suppressed by T cell vaccination, the inflammatory lesions may still take a long time to resolve and some of the existing tissue damage will be permanent. Moreover, in some patients with advanced disease, the inflammatory lesions may not be directly associated with myelin-reactive T cell responses. Consequently, depletion of MBP-reactive T cells may have little impact on the disease processes in these patients. It is hoped that further investigations may provide new insights into our understanding of these fundamental issues.

The findings described here regarding the treatment efficacy of T cell vaccination should be interpreted with

caution because of the inherent limitations of the study. In the absence of placebo controls, the clinical results were compared with the patient's own pre-treatment status. Such comparisons may introduce biases in the interpretation of the results. The study is also limited by the potential placebo effect associated with the open-label design of the study. Therefore, although the study provided important clinical indications in favor of the potential role of T cell vaccination in MS, the treatment efficacy of T cell vaccination must be evaluated in double-blind and placebo-controlled clinical trials.

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